# Project Report: Microbial diversity and population structure studies in the Rio Tinto

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## Project Progress

As part of our *Microbial diversity and population structure studies in the Río Tinto* , we have carried out two full sampling efforts, in the fall of 2003 and in January of 2004, corresponding to the dry and wet season extremes. We have chosen our stations based on varying concentrations and oxidation state of iron present (see http://astrobiology.mbl.edu/riotinto/ for more detailed information and photographs of the study locations). For each station we have sampled three different sites, with three–fold replication. For each sample, we filtered 2 L of water for DNA extraction and concomitantly collected physico–chemical parameters consisting of *in situ* (pH, oxygen, redox, temperature and conductivity) and *ex situ* (S, Fe, Zn, Cu, Al, As, Ni, Mg, Ca, K, etc.) measurements, and preserved formalin samples for bacterial and protist enumeration. To date, all DNA has been extracted and physico–chemical measurements completed. We plan to use these DNA samples to both build rDNA clone libraries and conduct a three–domain serial analysis of ribosomal sequence tags (3D–SARST) analysis for the three domains of life.

We are currently analyzing the results of both clone library sequences and serial analysis of V6 ribosomal sequence tags (SARST–V6) hypervariable region– a region spanning from 52–152 base pairs in length in known bacteria) experiments on samples collected during a pilot project conducted in October of 2002. We have built bacterial, archaeal and eukaryal clone libraries for one sample from each station. Over seventy unique clones were fully sequenced and used in a three–domain phylogenetic analysis consisting of 300 total taxa. Over 4,000 SARST sequenced tags have also been recovered and used in calculating alpha and beta diversity indices. The SARST diversity data will be combined with the physico–chemical parameters in analyses that will reveal important aspects of the biogeochemistry of this extreme environment. We will summarize these data in a review paper of the diversity in the river and other publications focusing on the microbial ecology to be submitted later this year.

## Highlights

- Phylogenetic analyses of full–length sequences revealed additional phylotypes not detected in previous studies including members of the

archaeal “alphabet” plasmas, bacterial sequences related to environmental sequences from other acidic extreme environments, and sequences derived from relatives of anaerobic amoeboid protists.
- SARST−V6 found many of the same bacteria in the Río Tinto as reported using other microbial ecology methods, however, also identified organisms never detected in this extreme environment before such as free−living bacteria, parasites, and endosymbionts.
- Some of the most abundant SARST−V6 sequences matched 100% several other uncultured bacterial organisms not reported in the Río Tinto before but observed in other acidic environments like Iron Mountain.

## Roadmap Objectives

- ***Objective No. 5.1:*** Environment−dependent, molecular evolution in microorganisms
- ***Objective No. 5.2:*** Co−evolution of microbial communities
- ***Objective No. 5.3:*** Biochemical adaptation to extreme environments

## Field Expeditions

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| ***Field Trip Name:*** Rio Tinto Sampling | |
| ***Start Date:*** 25 Sept 03 | ***End Date:*** 27 Sept 03 |
| ***Continent:*** Europe | ***Country:*** Spain |
| ***State/Province:*** Andalucia | ***Nearest City/Town:*** Nerva |
| ***Latitude:*** N 37degrees 43.316 minutes | ***Longitude:*** W 06 degrees 33.067 minutes |
| ***Name of site(cave, mine, e.g.):*** Origin, Anabel's Garden, Berrocal | ***Keywords:*** |
| ***Description of Work:*** Collected water samples for DNA and RNA extraction; measured in situ pH, oxygen, conductivity, redox and temperature; also ex situ geochemical analsyes of major metals using TXRF and ICP methods; collected samples for formalin preservation; | |
| ***Members Involved:*** | |

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| ***Field Trip Name:*** Rio Tinto Sampling | |
| ***Start Date:*** 03 Jan 04 | ***End Date:*** 07 Jan 04 |
| ***Continent:*** Europe | ***Country:*** Spain |
| ***State/Province:*** Anadulcia | ***Nearest City/Town:*** Nerva |
| ***Latitude:*** N 37degrees 43.316 minutes | ***Longitude:*** W 06 degrees 33.067 minutes |
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***Members Involved:***

## Cross Team Collaborations

CAB member Ricardo Amils on field related work described above. A review paper is in progress, as well as the first manuscript from the SARST work.